1992

Determination of cyanogenesis and total cyanide in deoiled jojoba meal

Ketan Desaibhai Patel
San Jose State University

Order Number 1348709

Determination of cyanogenesis and total cyanide in deoiled jojoba meal

Patel, Ketan Desaibhai, M.S.

San Jose State University, 1992

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

DETERMINATION OF CYANOGENESIS AND TOTAL CYANIDE IN DEOILED JOJOBA MEAL

A Thesis

Presented to

The Faculty of the Department of Nutrition and Food Science

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in Nutritional Science

By

Ketan Desaibhai Patel

May 1992

APPROVED FOR THE DEPARTMENT

OF NUTRITION AND FOOD SCIENCE

Dr. Panfilo Belo

Dr. Miriam Saltmarch

Dr. Lucy McProud

APPROVED FOR THE UNIVERSITY

Serena N. Stanford

ABSTRACT

DETERMINATION OF CYANOGENESIS AND TOTAL CYANIDE IN DEOILED JOJOBA MEAL

by Ketan D. Patel

Liberation of cyanide (cyanogenesis) and total cyanide content of deoiled meal prepared from whole jojoba seeds and from pressed cake residue obtained from commercial jojoba oil processing plant were determined. Cyanogenesis tests carried out in semimacro and macro diffusion set-ups at room temperature. Under aqueous conditions, about 6 and 11 ppm of cyanide (on dry weight basis) were liberated after 24 and 48 hr at room temperature, respectively. Deoiled meal maintained under acid conditions liberated higher cyanide than that maintained under aqueous and phosphate buffer (pH 7.0) conditions. Cyanogenesis tests under acid conditions carried out for 33 days at room temperature showed a relatively slow liberation rate of cyanide. Combined alkali and heat treatment of water extract prior to color development showed positive reaction to cyanide comparable with that of pure simmondsin. Total cyanide content of deoiled jojoba meal determined by this method ranged from 1121 to 1548 ppm.

ACKNOWLEDGEMENTS

I wish to acknowledge my graduate advisor, Dr. Panfilo Belo for his time, patience and advice throughout all phases of this research; Dr. Miriam Saltmarch and Dr. Lucy McProud for guidance and assitance with manuscript organization. I thank Mr. Bob Caron, Ms. Louella Demers and Mrs. Judith Schallberger for their timely help in the laboratory. I wish to acknowledge my parents, wife and family for their continued encouragement and support throughout the course of my studies at San Jose State University.

PREFACE

The following is a publication style thesis. The second chapter is written in journal format and will be submitted to the Journal of Agricultural and Food Chemistry. Chapter I and III are written according to guidelines outlined in the Publication Manual of the American Psychological Association, 3rd. edition, 1986.

Table of Contents

List of Figures

List of Tables

Table Page

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Jojoba (*Simmondsia chinensis*) is a dioecious plant that grows naturally on arid and semi-arid lands in Arizona, California, Mexico and other areas of the world (NAS, 1975, 1977). Interest in commercial propagation of this plant started in the 1970's when the United States government banned the importation of sperm whale oil to protect the endangered animal (NAS, 1975). The economic value of jojoba oil as a substitute for sperm whale oil is now well recognized (NAS, 1985). At present jojoba is commercially propagated for its oil in various areas of the world. In 1985, around 16,000 hectars of semi-arid and arid lands in southwestern U.S. have been cultivated for commercial production of jojoba oil (NAS, 1985).

Jojoba seeds contain on the average about 50% by weight of oil which is similar to sperm whale oil in chemical and physical properties (NAS, 1975, 1977, 1985; Yermanos & Duncan, 1976). Chemically, jojoba oil is a liquid wax which is composed mostly of straight chain mono-esters of long chain mono-ethylenic alcohols and fatty acids. This is in contrast to vegetable or animal oils which are triglycerides (Wisniak, 1977; NAS, 1975, 1977). Studies indicate that jojoba oil can duplicate sperm whale oil in many applications (NAS, 1975, 1977).

Both solvent and mechanical extraction are currently used in the

commercial production of jojoba oil. Jojoba meal, which represents about 50% by weight of the whole seed, is the residue remaining after oil extraction. At present, this by-product of the jojoba oil industry has no commercial uses. Because of the relatively high amount of protein in the meal (20-34%), studies on its utilization have been focused on its potential as a feed ingredient for livestock and poultry. However, adverse effects in feed intake, feed efficiency, growth and overall nutritional status have been observed in chicken, sheep, cattle and laboratory test animals (mice, rats,etc) when fed with rations containing up to 10% deoiled jojoba meal (Booth et al., 1974; Verbiscar et al., 1978, 1980, 1981). These adverse effects have been attributed to the presence of a group of closely related cyanogenic glycosides present in jojoba seed. Simmondsin and simmondsin 2' ferulate are the two major cyanogenic glycosides found in jojoba (Booth et al., 1974; Verbiscar et al., 1980).

Cyanogenic glycosides are widely distributed in the plant kingdom. This group of naturally occurring toxicants has been identified in more than 1000 species of plants (Conn, 1969). Major cyanogenic glycosides which are important to human and animal nutrition include linamarin, amygdalin, prunasin and dhurrin. Linamarin is found in whiteflex (linseed), cassava, and lima beans. Amygdalin is present in Rosaceae, such as choke cherries, wild cherries, mountain mahogany, saskaton, service berries and kernels of almonds, apricots, peaches and apples. Prunasin is also found in these

plants; it has the same structure as amygdalin except it has one glucose rather than two attached to the aglycon. Dhurrin occurs in grain sorghums, forage sorghums and johnson grass (Conn, 1969). Cyanogenic glycosides are toxic because they liberate cyanide or prusic acid upon hydrolysis. Cyanide is a potent respiratory inhibitor which inhibits cytochrome oxidase resulting in oxygen starvation at the cellular level (Conn, 1969).

The hydrolysis of cyanogenic glycosides by endogenous enzyme results when the plant tissue is crushed or disrupted. The enzyme which hydrolyzes the glycoside is present in the same plant, but apparently in different cell compartments. When the plant tissue is crushed, the endogenous enzyme and cyanogenic glycoside come into contact with each other, resulting in the releases of cyanide. This enzymatic hydrolysis is the basis of detoxification of linamarin, prunasin, amygdalin and dhurrin (Tinay et al., 1984). In cassava (*Manihot esculenta*), a major staple food in the tropics, the liberation of cyanide or cyanogenesis is enhanced during processing (Conn, 1969).

It is apparent that cyanogenic glycosides have one property in common, that is, their ability to release cyanide as a product of disintegration. Since the formation of cyanide has been identified as the toxic factor of cyanogenic plants, it has been the main focus of analytical determination of cyanogenic compounds. While methods for cyanide analysis have been

developed and used in the determination of the toxicity of cyanogenic plants or products derived from these plants, their application to jojoba meal has not been studied.

Objectives

The objectives of this study were to determine the cyanide potential in whole jojoba seed and defatted jojoba meal and to develop a macro diffusion method to detect cyanide at lower levels. The effect of acid on the liberation of cyanide and alkali-heat treatment on the determination of total cyanide content of whole seed and deoiled jojoba meal were studied and compared with cassava and almond meal.

Significance of the Study

Since jojoba meal constitues about half of the whole seed, commercial jojoba oil processing operations must consider handling the large amount of this by-product and its potential use. Currently, the defatted jojoba meal is discarded because it contains cyanogenic glycosides which have been shown to be toxic to rats, mice and ruminants. In order to assess the utilization of this by-product for animal and human consumption, studies on hydrolysis of cyanogenic glycosides and determination of cyanide are needed. Measurement of cyanogenesis or content of cyanide is essential for control of the cyanide because of the toxicity risk, for scientific purposes such as studies of chemotaxonomy or breeding of jojoba for low cyanide, and for development of detoxification processes. No studies have been

done to determine the amount of cyanide in deoiled jojoba meal.

Review of Literature

Jojoba

Jojoba (*Simmondsia chinensis*) is a desert shrub that grows wild in Southwestern United States, Mexico, Latin America, Israel, South Africa, Australia and other African nations (NAS, 1975, 1977, 1985). It is a woody evergreen plant which grows to 10-15 feet in height and easily recognized by its thick, leathery, bluish-green leaves and dark brown nut-like fruit (NAS, 1975). Jojoba is resistant to drought and does not seem to be subjected to serious insect attack or disease damage (NAS, 1975). Jojoba is a dioecious plant, the male and female flowers are born on separate plants. The female plant bears the fruit which contains the seeds (Wisniak, 1977). Commercial propagation of jojoba in the U. S. started in the 1970's when the U.S. government banned the importation of sperm whale oil to protect the endangered animal (NAS, 1975, 1977). Currently, about 259,000 square km of arid land in the Sonoran desert of Mexico and the United States have been planted commercially and the trade of jojoba oil products and 11,000 to 18,000 tons of dry seeds each year is now a multimillion doller industry (Hinman, 1986; NAS, 1985; Ngoupayou, 1982).

Mature Jojoba seeds contain on the average 50% by weight of oil which resembles spermwhale oil in chemical and physical properties (NAS, 1975). Jojoba oil is not triacylglycerol but a liquid wax which is composed

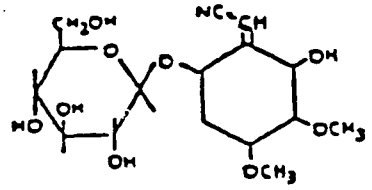
of esters of high molecular weight, straight chain mono-ethylenic acids and mono-ethylenic alcohols and fatty acids (NAS, 1975). Jojoba oil is highly resistant to oxidative and hydrolytic rancidity, and repeated heating at high temperatures (NAS, 1977). The viscosity, flash, smoke and fire points are similar to that of spermwhale oil (NAS, 1977). Hydrogenation of the oil gives a hard white wax which is similar to beeswax, candellia, carnuba and spermaceti waxes (NAS, 1977). It is also used in cosmetics and as high pressure lubricants, transformer fluids, coatings and stabilizing agents in penicillin. Potential uses include cooking and vegetable oil, shortening and in salad oil as a low caloric additive (NAS, 1977).

Defatted jojoba meal constitutes about 50% by weight of the whole seeds (NAS, 1975). It contains about 30% crude protein and 8% total sugars which make it a potential feed ingredient for livestock (Verbiscar et al., 1980). The amino acid composition of deoiled jojoba meal has been studied (NAS, 1975; Verbiscar & Banigan, 1978). It has a very high amount of lysine but a very low amount of methionine (0.6-1.2%) (Verbiscar & Banigan, 1978; Yermanos & Duncan, 1976). Studies on functional properties (Cardoso & Price, 1980; Wiseman & Price, 1987) showed that water and oil absorption capacities of jojoba meal protein were comparable to that of soy protein concentrates, and foaming capacity and stability were also similar to those of egg albumin.

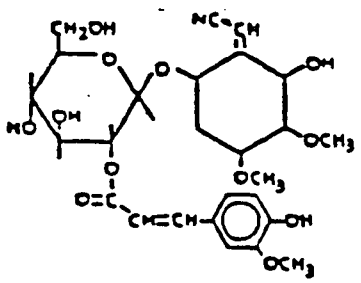
At present, jojoba meal has no commercial uses. The animal feeding

studies indicated adverse effects on total food intake, food digestibility and efficiency growth, and overall nutritional status of chickens, sheep, cattle and lamb fed rations containing up to 10% deoiled jojoba meal (Verbiscar et al., 1978, 1980, 1981; Ngoupayou, 1982; Verbiscar & Banigan, 1983). These adverse effects have been attributed to the presence of cyanogenic glycosides. The major cyanogenic glycosides identified in jojoba seeds include simmondsin and simmondsin 2′ ferulate (Figure 1) (Booth et al., 1974). Jojoba meal contains 3.6 to 6.0 percent simmondsin and 0.35 to 2.0 percent simmondsin 2′ ferulate (Booth et al., 1974). The acute toxicity studies in rats by Booth et al. (1974), Verbiscar et al. (1980), and Manos et al. (1986) showed that dietary simmondsin at 0.3% or greater caused appetite depression and weight loss. Successive oral doses of 750 mg/kg body weight of simmondsin for five days to young adult mice resulted in mortality within 10 days after the first dose. However, a single large dose of 4 gm/kg produced no apparent ill effects. The authors believed that the toxicity of simmondsin may have been due to its metabolic conversion to a benzyl cyanide derivative in the animal body (Booth et al., 1974). The subcutaneous lethal dose for benzyl cyanide in mice was found to be 32 mg/kg (Booth et al., 1974).

Manos et al. (1986) reported that residues of simmondsin and simmondsin 2′ ferulate were not found in the blood, kidney, liver or muscle of lambs fed rations containing 5 to 10% jojoba meal. This plus the fact



Simmondsin



Simmondsin 2′ ferulate

Figure 1. The structure of simmondsin and simmondsin 2′ ferulate.

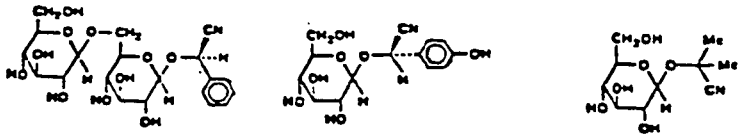
that lower weight gain and feed intake and efficiency were observed led these authors to hypothesize that the cyanogenic glycosides in jojoba are detoxified by microorganisms in the gut of the ruminants.

Cyanogenic Glycosides and Cyanogenesis

A wide variety of plants contain compounds with cyano groups which contribute to the toxicity of the plant material for food or feed use. Some of these compounds, termed cyanogenic glycosides, liberate cyanide, sugar and an aldehyde or ketone on treatment with dilute acid or the appropriate hydrolytic enzymes (Conn, 1969). This ability of plants to produce cyanide, known as cyanogenesis, has been recognized in cassava, apricots, peaches, almonds and other important food plants (Seigler, 1975).

Approximately 1000 plant species have been reported to be cyanogenic. Major cyanogens of importance in human or animal nutrition

are amygdalin (laetrile), dhurrin and linamarin. The structure of these compounds as reported by Conn (1969) is shown in Figure 2. Amygdalin is found in Rosaceae, such as choke cherries, wild cherries, mountain mahogany, saskaton service berries and the kernels of almonds, apricots, peaches and apples. Dhurrin occurs in sorghum species such as grain sorghums, forage sorghum, and johnson grass. Linamarin is found in white flax (lineseed), cassava, and lima beans (*Phaseolues lunatus c.*) (Conn, 1969). Amygdalin is a beta-glycoside of D(-) mandelonitrile or benzaldehyde cyanohydrin. On hydrolysis, this glycoside yields glucose, benzaldehyde, and cyanide. Dhurrin is the aglycone of p-hydroxymandelonitrile. The hydrolysis of dhurrin gives glucose, benzaldehyde and cyanide. Linamarin, acetone cyanohydrin beta D-glycoside, on hydrolysis produced glucose, acetone and cyanide (Conn,1969). Many poisonings have been caused by the ingestion of these and other plant sources. The toxicity is a result of the hydrolysis of the glycoside and subsequent release of cyanide into the body of the victim (Cotgageorge, 1978). Cyanide is a potent respiratory inhibitor, and the site of inhibition is the enzyme cytochrome oxidase, the terminal respiratory catalyst of aerobic organisms. Cyanide poisoning, therefore, results in death due to oxygen starvation at the cellular level. Death usually follows ingestion of a lethal dose within 15 minutes to a few hours. Two hundred mg of cyanide is a lethal dose for adult humans. Plants which contain more than 20 mg cyanide per 100 gm tissue can be considered



Amygdalin Dhurrin Linamarin

Figure 2. The structure of major cyanogenic glycosides from plants.

potentially dangerous (Cotgageorge, 1978). Serious side effects from sustained, sub-lethal doses are commonly observed in those populations whose staple food source is manioc (cassava). The edible root of this plant is rich in carbohydrate and the source of commercial tapioca. People generally scrape the roots and soak them in water to ferment for several days. Under these conditions cyanogen gets extensively hydrolyzed till both it and its hydrolysis products are leached out (Conn, 1971). Hydrolysis and subsequent release of cyanide has been used as a detoxification technique in cassava (Maduagwu & Umoh, 1982; Tinay et al., 1984), bitter almond (Nashida et al., 1988), and sorghum species (Haskins et al., 1988) but no studies have been done on jojoba.

Detoxification of Jojoba Meal

Various approaches for the detoxification of jojoba meal have been reported (Elliger et al., 1975; Banigan & Verbiscar, 1980; Verbiscar et al., 1980, 1981). Solvent extraction, water washing, heat, chemical treatments and microbial methods resulted in reduced simmondsin levels (Verbiscar et

al., 1980, 1981). Verbiscar et al. (1980) reported that water extraction reduced the simmondsin and simmondsin 2′ ferulate contents of jojoba meal to 0.08 percent. The most effective chemical detoxification process using ammonium hydroxide and hydrogen peroxide has been studied (Banigan & Verbiscar, 1980). These chemicals reduced simmondsin leveis to 0.19 percent and simmondsin 2′ fertulate levels to 0.08 percent or less. Verbiscar et al. (1981) studied microbial treatment as a detoxification process. They found that selected strains of *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Saccharomyces cerevisae* grew well on jojoba meal and reduced the levels of simmondsin and simmondsin 2′ ferulate. Growth of Lactobacillus acidophilus B629 in defatted jojoba meal reduced the total toxicants by 95 to 98 percent (Verbiscar et al. 1981).

Determination of Cyanide or HCN

The measurement of cyanide levels is of great importance to the food technologist anxious to develop processing methods that would yield edible zero cyanide plant products, to the plant breeder interested in breeding low cyanide plant cultivers and the toxicologist who may need to assess the extent of toxicity by monitoring blood levels of free cyanide and its biotransformation products (Ikediobi et al., 1980).

Several methods for the determination of cyanide have been studied in cassava (Mendoza et al., 1984), soybeans and soybean products (Honig et al., 1983), apricot kernels (Egli, 1977), animal feeding stuffs (Harris et al.,

1980), and dried sorghum leaves (Haskins et al., 1988). All of these methods are based on the assay of cyanide released when the cyanogenic glycoside is chemically or enzymatically hydrolyzed to yield cyanide, glucose and aglycone.

Lambart et al. (1975) introduced stable reagents for the colorimetric determination of cyanide by modified Konig reactions. This specific and quanitative reaction has been applied to the determination of cyanide by oxidation of CN^- to a cyanogen halide, CNX. The CN^+ then reacts with pyridine to produce an intermediate which hydrolyzes to a glutaconic aldehyde. The glutaconic aldehyde is coupled with a primary amine or a compound containing reactive methylene hydrogens to produce a colored species.

Nahestedt et al. (1981) proposed methods of liberating and estimating cyanide from cyanogenic plant material. It involves three steps: 1) liberation of cyanide from cyanogenic plant material by acid or endogenous or exogenous enzyme; 2) isolation and concentration of the liberated cyanide into a trap; and 3) estimation of the isolated cyanide using chemical reactivity or physical behavior of the cyanide ion. Some researchers (Nashida et al., 1987; Rubio et al., 1987; Harris et al., 1980; Mendoza et al., 1984) isolated cyanide by diffusion in a closed system like the Conway's semimicro diffusion apparatus (Figure 3) from one comportment containing the sample into another comportment containing the alkaline trap.

Some authors (Gondive, 1974; Wokes & Willimont, 1951) reported the cleavage of cyanogenic glycosides by dilute acids (Nahrstedt et al., 1981). Release of HCN by acid hydrolysis was studied in soybean and soybean products (Honig et al., 1983). The method involved acid hydrolysis of the sample with diffusion or distillation and collection of the liberated cyanide in sodium hydroxyide solution and determination of cyanide by pyridine-barbiturate acid colorimetric procedure.

Mendoza et al. (1984) compared three spectrophotometric methods for the analysis of cyanide in cassava root extracts, namely pyridine-barbiturate, pyridine-pyrazolone and alkaline picrate methods. Among these the pyridine-barbiturate method was found to be the best due to sensitivity, specificity, stability of reagents, short time of analysis (10-15 minutes), stability of color and simplicity of analysis.

A colorimetric procedure for the determination of cyanide in enzyme-hydrolyzed extracts of dried sorghum leaves by alkali hydrolysis has been studied (Haskins et al., 1988). The method involved drying of the tissue at 75° C, grinding the dry tissue, extracting it with water, digesting the extract with alkali to release cyanide and determining cyanide colorimetrically by using pyridine-barbiturate reagent.

At present, there are no available data on the cyanide content of whole jojoba seed and residue remaining after oil extraction. This study will

attempt to determine the amount of cyanide released from jojoba meal on the hydrolysis with acid and combined alkali-heat treatments.

CHAPTER 2

JOURNAL ARTICLE

Authors Title Page

DETERMINATION OF CYANOGENESIS AND TOTAL CYANIDE IN DEOILED JOJOBA MEAL

Ketan D. Patel, Panfilo S. Belo*, Miriam Saltmarch, and Lucy McProud

Department of Nutrition and Food Science

San Jose State University

1 Washington Square

San Jose, CA 95192

* Address question and comments to: Dr. Panfilo Belo,

Department of Nutrition and Food Science, San Jose State University,

1 Washington Square, San Jose, CA 95192, 408-924-3108.

ABSTRACT

Liberation of cyanide (cyanogenesis) and total cyanide content of deoiled meal prepared from whole jojoba seeds and from pressed cake residue obtained from commercial jojoba oil processing plant were determined. Cyanogenesis tests carried out in semimacro and macro diffusion set-ups at room temperature. Under aqueous conditions, about 6 and 11 ppm of cyanide (on dry weight basis) were liberated after 24 and 48 hr at room temperature, respectively. Deoiled meal maintained under acid conditions liberated higher cyanide than that maintained under aqueous and phosphate buffer (pH 7.0) conditions. Cyanogenesis tests under acid conditions carried out for 33 days at room temperature showed a relatively slow liberation rate of cyanide. Combined alkali and heat treatment of water extract prior to color development showed positive reaction to cyanide comparable with that of pure simmondsin. Total cyanide content of deoiled jojoba meal determined by this method ranged from 1121 to 1548 ppm.

INTRODUCTION

The economic value of jojoba (*Simmondsia chinensis*, also known as *Simmondsia california*) as a source of liquid wax which resembles sperm whale oil is now well recognized (NAS, 1976, 1977, 1985). Ever since sperm whale oil importation was banned in the U.S. in the 1970's, a great deal of effort has been directed toward the commercial cultivation of this wild desert plant both here and abroad. In 1985, about 16,000 hectares of semi-arid and arid lands in Southwestern U.S. have been cultivated for commercial production with an estimated value of about two hundred million dollars (NAS, 1985).

Mature jojoba seeds contain on the average 50% by weight of oil which resembles sperm whale oil in chemical and physical properties (NAS, 1975). Jojoba oil is not a triacylglycerol but a liquid wax which is composed of esters of high molecular weight, straight chain mono-ethylenic acids and mono-ethylenic alcohols and fatty acids (NAS, 1975). Jojoba oil is highly resistant to oxidative and hydrolytic rancidity, and repeated heating at high temperatures (NAS, 1977). The viscosity, flash, smoke and fire points are similar to that of spermwhale oil (NAS, 1977). Hydrogenation of the oil gives a hard white wax which is similar to beeswax, candellia and spermaceti waxes (NAS, 1977). It is also used in cosmatics and as high pressure lubricants, transformer fluids, coatings and stabilizing agents in penicillin. Potential uses include cooking and vegetable oil, shortening, and

in salad oil as a low caloric additive (NAS, 1977).

Both solvent and mechanical extraction are currently used in the commercial production of jojoba oil. Jojoba meal, which represents about 50% by weight of the whole seed, is the residue remaining after oil extraction. At present, this by-product of the jojoba oil industry has no commercial uses. Because of the relatively high amount of protein in the meal (20-34%), studies on its utilization have been focused on its potential as feed ingredients for livestock and poultry. However, adverse effects in feed intake, feed efficiency, growth, and overall nutritional status have been observed in chicken, sheep, cattle and laboratory test animals (mice, rats) when fed rations containing 10% deoiled jojoba meal (Booth et al., 1974; Verbiscar et al., 1978, 1980, 1981). These adverse effects have been attributed to the presence of a group of closely related cyanogenic glycosides present in jojoba seed. Simmondsin and simmondsin 2' ferulate are the two major cyanogenic glycosides found in jojoba (Booth et al., 1974; Verbiscar et al., 1980).

Cyanogenic glycosides are widely distributed in the plant kingdom and have been identified in more than 1000 species of plants (Conn, 1969). This group of naturally occurring compounds are potentially toxic because of their ability to liberate cyanide upon hydrolysis. Cyanide is a potent respiratory inhibitor which inhibits cytochrome oxidase, resulting in oxygen starvation at the cellular level (Conn, 1969). Liberation of cyanide can be

brought about by endogenous enzyme or by the enzymes present in the digestive tract.

While the mechanism of the toxic action of simmondsin is still unknown, animal toxicity studies indicate that simmondsin must be metabolized, possibly by bacteria in the digestive tract to be toxic since intraperitoneal administration did not produce symptoms of toxicity (Booth et al., 1974). It is postulated that the toxicity of simmondsin might be due to the formation of the phenyl acetonitrile derivative which is generally metabolized to cyanide.

Since the formation of cyanide has been identified as the toxic factor of cyanogenic plants, it has been the main focus of analytical determination of cyanogenic compounds. While methods for cyanide analysis have been developed and used in the determination of the toxicity of cyanogenic plants or products derived from these plants, their application in jojoba seeds and meal has not been studied. Measurement of cyanogenesis or cyanide content is essential for control of cyanide because of the toxicity risk, for scientific purposes such as studies of chemotaxonomy or breeding of jojoba for low cyanide, and for development of detoxification processes.

The objective of this study was to determine cyanide potential of deoiled jojoba meals prepared from whole seed and residue obtained from commercial jojoba oil processing plant. A macro diffusion method for the detection of cyanide at low levels was developed. The effect of acid on the

liberation of cyanide and alkali-heat treatment on the determination of total cyanide content of deoiled jojoba meal were determined and compared with that of cassava and almond meals.

MATERIALS AND METHODS

Sample and Sample Preparation. The deoiled jojoba meal samples used in this study were prepared from whole seed, meal and pressed cake samples using Soxhlet apparatus and hexane as the extracting solvent. Mature jojoba seeds and meal samples were obtained from Sigma Chemical Company, St Louis, MO. Pressed cake samples were obtained from commercial jojoba oil processing plant (Desert Whale Jojoba Company, Tucson, AZ). Pressed cake is the residue remaining after mechanical oil extraction. The deoiled samples were air dried and ground prior to use.

Cassava tubers were purchased from a local grocery market. Almond meal was obtained from Sigma Chemical Company. Pure simmondsin was obtained from Dr. Anthony J. Verbiscar, Anver Bioscience Design, Inc., Sierra Madre, CA.

Proximate Analysis. Analysis for crude protein, crude fat, ash and moisture were performed on deoiled samples using the methods of the Association of the Official Analytical Chemist (AOAC, 1984).

Determination of Cyanogenesis. Preliminary study on the liberation of hydrocyanic acid (cyanogenesis) from deoiled jojoba meal, cassava and almond meal were carried out in Conway's semimicro diffusion apparatus

(Figure 3). Because of the limited amount of sample that can be used in microdiffusion apparatus, subsequent cyanogenesis tests were carried out in semimacro (Figure 4) and macro (Figure 5) diffusion set-ups. Two to 5 g and 10 to 20 g samples were used in semimacro and macro diffusion set ups, respectively. Three ml, and 5 to 10 ml of cyanide absorbing solution (1.0 N NaOH) were used in semimacro- and macro-diffusion set-ups, respectively.

The amount of cyanide absorbed in the NaOH solution was determined colorimetrically using the pyridine-barbituric acid method (Lambert et al., 1975) as modified by Haskin et al. (1988). Aliquot (0.1 to 1.0 ml) of NaOH was treated with 0.5 ml of 10 N acetic acid followed by 5 ml of solution containing 2.5 g of succinimide and 0.25 g of N-chlorosuccinimide in 1 L of water. The color was developed by adding 1 ml of solution containing 6 g barbituric acid, 30 ml of pyridine and 70 ml of water. The mixture was allowed to stand for 20 min. at room temperature and absorbance was read at 580 nm against 1 N NaOH plus the color reagents as blank. Amount of cyanide liberated was determined from a standard curve prepared from 1 x 10^{-4}M KCN.

The presence of cyanide in the absorbing solution was further confirmed by taking spectrophotometric spectra from 200 to 400 nm. against 1.0 N NaOH using UV 160 Shimadzu recording spectrophotometer. The wavelength corresponding to the maximum absorption for cyanide was

obtained.

Cyanogenesis in Jojoba Meal, Cassava and Almond Meal Under Aqueous Condition. Three gram samples of deoiled jojoba meal was placed in semimacro diffusion apparatus (Figure 4). Ten ml of distilled water was added and mixed thoroughly. The amount of cyanide liberated was compared with that of almond meal and freshly grated cassava. Three gram and 20 g samples of almond meal and freshly grated cassava were used, respectively. Three ml of 1.0 N NaOH was used as cyanide absorbing solution. After 24 hr at room temperature, aliquots of NaOH solution were obtained and analyzed for cyanide.

Effect of Acid and Phosphate Buffer (pH 7.0) on Cyanogenesis. The purpose of this part of the study was to compare the effect of acid and buffer on the amount of cyanide liberated from deoiled jojoba meal. The tests were carried out in semimacro diffusion set up using 3 g sample of deoiled jojoba meal. Ten ml of distilled water, acid (1.0 N HCl) or 0.01 M phosphate buffer (pH 7.0) were added and the mixture was mixed thoroughly. Three ml of 1.0 N NaOH was used as cyanide absorbing solution. After 48 hr, the cyanide absorbing solution was scanned from 200-400 nm. Aliquots (1.0 ml) of this absorbing solution were obtained and analyzed for cyanide. The cyanide levels were calculated and expressed on a dry-weight basis.

Effect of Acid and Incubation Time on the Liberation of HCN from Deoiled Jojoba Meal. The effect of acid and incubation time on the liberation of cyanide from deoiled jojoba meal was verified and compared with almond meal and freshly grated cassava tuber using the macrodiffusion apparatus (Figure 5). Two 10-gram samples of deoiled jojoba meal were placed in the macrodiffusion apparatus. To further verify the liberation of cyanide, one of the samples was spiked with 26 microgram of cyanide (10 ml of 1 X 10^{-4} M KCN). After adding 20 ml of 1 N HCl the sample was mixed and the apparatus was sealed with 5 to 10 ml of 1.0 N NaOH as a cyanide absorbing solution. A similar set-up consisting of 10 g of almond meal and 25 g of freshly grated cassava was run along with the jojoba samples. The samples were incubated at room temperature and the amount of cyanide liberated was measured at various time intervals throughout 33-day incubation period. The cyanide absorbing solution was replaced with a fresh solution each time the cyanide level was assayed. The spectrophotometric scan (200-400 nm) of the absorbing solution was obtained after 24 hr incubation.

Effect of Alkali and Heat Treatment on the Formation of Cyanide. The effect of alkali and heat on the formation of cyanide in jojoba meal was determined using water extract. Typically, 1 g of sample was homogenized in 20 ml of distilled water using Potter Elvehjem homogenizer driven by electric drill. The extract was filtered and the 1.0 ml of the filtrate was

treated with 9.0 ml of 1.0 N NaOH and heated in boiling water. Aliquots were taken at various time intervals and analyzed for cyanide.

Comparision of Cyanide Formation Resulting from NaOH-Heat Treatment of Pure Simmondsin, and Simmondsin Isolate from Crude Extracts Prepared From Deoiled Jojoba Meal by TLC. To confirm the positive color reaction for cyanide obtained from the NaOH-heat treatment of the water extract, tests were conducted on pure simmondsin and crude isolates of simmondsin obtained from thin layer chromatography (TLC) of freeze dried water extract and dried acetone extract of deoiled jojoba meal. Acetone extraction and preparation of crude cyanoglycosides for TLC separation were carried out according to the procedure of Verbiscar and Banigan (1978). Five gram sample was subjected to 6 hour acetone extraction in Soxhlet extractor. The acetone extract was evaporated to dryness under vacuum and the residue was dissolved in 5.0 ml of ethanol. One ml aliquot of ethanol extract was mixed with 3.0 ml of ethyl acetate and was passed through a glass column (0.60 X 5.0 cm) containing 0.5 g of silica gel (230-400 mesh, Sigma Chem. Co.). The column was eluted with 10.0 ml of ethyl acetate:ethanol (7:3) mixture and the eluate which contained the cyanoglycosides was evaporated to dryness under vacuum. The residue was dissolved in 5.0 ml of methanol and the resulting extract was used in TLC separation. TLC was performed on silica gel precoated plates (20 X 20 cm, Sigma Chem. Co.) which was activated prior to use. Streak of the

methanol extract (200-500 ul) and pure simmondsin in methanol were applied and the plate developed using ethyl acetate-ethanol (7:3) mixture as the mobile phase. The separation was carried out until the solvent front was about 0.5 to 1.0 inch below the top edge of the plate. The plate was dried in the oven and the portion of the plate corresponding to the area where pure simmondsin and a small portion of the sample streak was sprayed with 10% sulfuric acid. Plate was heated in the oven at 100° C to develop the spots. The unsprayed streak corresponding to simmondsin was scraped and extracted with methanol four times using 10 ml of methanol each time. The extracts were combined and evaporated to dryness. The residue was dissolved in 1.0 ml of water and 9.0 ml of 1.0 N NaOH was added. The mixture was heated in boiling water bath for 2-4 minutes. After cooling to room temperature, the mixture was scanned from 200-400 nm. One ml aliquot was taken and reacted with cyanide color reagent as described previously. The spectrum of the extract in 1.0 N NaOH before heating and after heating were compared to that of the pure simmondsin. The spectrum of the color developed with cyanide reagent was also obtained and compared to that obtained with pure simmondsin.

The effect of NaOH-heat treatment on the determination of cyanide was further tested using crude isolate of cyanoglycosides prepared from the freeze dried water extracts of deoiled jojoba meal. Fifty grams of sample were extracted 4 times with 250 ml of water each time using a magnetic

stirrer. The filtrates from each extraction were combined and freeze dried.

Freeze dried water extract (0.40g) was extracted with 3.0 ml methanol, filtered and the resulting filtrate was used in TLC separation of cyanoglycosides. TLC separation was carried as described above. Streak on the TLC plate corresponding to simmondsin was isolated, reacted with 1.0 N NaOH, analyzed spectrophotometrically before and after heating, and analyzed for cyanide as described previously. Results were compared with that of pure simmondsin.

RESULTS AND DISCUSSION

Proximate Analysis. The results of proximate analysis performed on whole seeds (WJS) and meals obtained from Sigma Chem. Co. (JM-1) and from commercial jojoba oil processing plant (JM-2) are shown in Table I. With the exception of the crude protein content of whole jojoba seeds, the data for moisture, crude protein, crude oil (liquid wax), and ash are within the range of values reported for mature jojoba seeds and jojoba meal or residue remaining after oil extraction (Yermanos & Duncan, 1976; Verbiscar & Banigan, 1978; Ngoupayou, 1982). Whole seed contained on the average 11.4% crude protein on wet-weight basis. On dry weight and oil free basis, this crude protein value corresponds to 22.50% which is relatively low compared to the values reported (Yermanos & Duncan, 1976; Verbiscar & Banigan, 1978). Crude protein and crude oil contents of the jojoba meals used in this study ranged from 24 - 28.0%, and 1.0 - 7.0% (wet-weight

basis), respectively. On dry and oil free basis the crude protein correspond to 25.0 to 32.0%. These values are within the range of values reported in the literature (Verbiscar & Banigan, 1978; Verbiscar et al., 1980, 1981).

Liberation of Cyanide Under Aqueous Condition. Data presented in Table II show the amount of cyanide liberated from deoiled jojoba meal, freshly grated cassava and almond meal maintained under aqueous condition in diffusion apparatus for 24 hr at room temperature. On dry-weight basis the amount of cyanide liberated from deoiled jojoba meal was relatively lower than cassava, but slightly higher than almond meal. While liberation of cyanide from jojoba meal has not been reported, formation of cyanide from cassava and almond meal have been attributed to the hydrolysis of cyanogenic glycosides by the endogenous enzymes (Fukuba et al., 1984; Haisman & Knight, 1967). Since the liberation of cyanide from linamarin in cassava and from amygdalin in almond is now a well known fact, it is apparent from the results presented in Table II that cyanogenesis does occur in deoiled jojoba meal. However, whether the cyanide detected is the result of endogenous enzyme hydrolysis of the cyanogenic glycosides in deoiled jojoba meal remains to be determined.

Liberation of Cyanide Under Acid (1.0 N HCl), Phosphate Buffer (pH 7.0), and Aqueous Conditions. With the use of the diffusion set-up, the effect of acid, phosphate buffer (pH 7.0) and water on the liberation of cyanide were compared. Phosphate buffer (0.01 M) was used to maintain

the pH of the deoiled meal throughout the incubation period. Phosphate buffers have been used to study the enzymatic hydrolysis of cyanogenic glycosides in cassava (Mendoza et al., 1984). The amount of cyanide liberated after incubation for 48 hr at room temperature are presented in Table III. The liberation of cyanide from deoiled jojoba meal under aqueous medium observed in the previous test (Table II) was confirmed. Increasing the incubation time at room temperature from 24 to 48 hours almost doubled the amount of cyanide liberated. However, it should be noted that the incubation was terminated after 48 hours due to considerable amount of mold growth on deoiled meal samples maintained in aqueous and phosphate buffer medium. Samples treated with 1.0 N HCl showed the highest amount cyanide liberated and did not show any sign of mold growth after 48 hr incubation. Based on the amount of cyanide liberated, maintaining deoiled jojoba meal in phosphate buffer did not enhance the liberation of cyanide.

Figure 6 shows the spectra of the 1.0 N NaOH used as cyanide absorbing solution in the above diffusion tests. All three solutions showed maximum absorbance at 224 nm. Addition of acetic acid shifted the maximum absorbance peak to 242 nm. Addition of acetic acid is the first step in the colorimetric determination of cyanide absorbed in the NaOH. It is postulated that the maximum absorption observed at 224 nm is due to the nitrile chromophore present in cyanide compound liberated from deoiled jojoba meal samples. Pure KCN in 1.0 N NaOH gave the same absorption

spectrum with maximum absorption peak at 224 nm (not shown in the figure). Addition of acetic acid, however, did not result in the shift of the maximum absorption but rather the disappearance of the maximum absorption peak. Since the formation of gas bubbles was noticeable upon addition of acetic acid, it is presumed that the disappearance of the peak in pure KCN is the result of the formation of HCN which is lost upon standing. Because of the absence of mold growth and relatively high amount of cyanide liberated resulting from acid treatment, further tests were conducted using larger sample size and longer incubation time.

Effect of Acid (1.0 N HCl) on Cyanogenesis in Deoiled Jojoba Meal, Cassava and Almond Meal. Data presented in Figure 7 show the comparison of the cumulative amount of cyanide liberated from 10 grams of deoiled jojoba meal samples treated with 20 ml of 1.0 N HCl, with and without pure KCN added, and incubated at room temperature for 33 days. The sample spiked with 26 microgram of cyanide (1.0×10^{-3} mmole KCN) showed a higher amount of cyanide liberated throughout the 33 days incubation. On a dry weight basis, 97 and 70 ppm total cyanide were liberated from deoiled jojoba meal with and without pure KCN added, respectively. While almost all of the added cyanide was recovered during the 33-day incubation period, the liberation of cyanide was relatively slow. Although it is apparent from Figure 7 that the maximum total cyanide of the two samples was not attained, the liberation of cyanide resulting from acid

treatment is apparent.

Spectrophotometric scan of the cyanide absorbing solution (1.0 N NaOH) from the above test are shown in Figure 8. After 24 hr incubation, the two cyanide absorbing solutions showed similar maximum absorption peaks at 224 and 242 nm before and after addition of acetic acid, respectively. Spiking the sample with 26 micro gram of cyanide, resulted in the slight increase in absorbance at 224 nm and slight decrease in absorbance at 242 nm. These results confirmed previous data (Figure 6) which indicate that the 224 peak is due to the cyanide liberated from deoiled jojoba meal.

Figure 9 compares the amount of cyanide liberated from deoiled jojoba meal, freshly grated cassava and almond meal during the 30 day incubation period at room temperature. The increase in cyanide liberated during the 30-day incubation period was highest in cassava followed by deoiled jojoba meal and only very slight increase in almond meal. Judging from the slope, it is apparent that the release of cyanide from jojoba is relatively slow compared to cassava, but faster than the almond meal. After 30 days, about 190, 70 and 12 ppm of cyanide (dry-weight basis) where liberated from freshly grated cassava, deoiled jojoba meal and almond meal, respectively. Since it is apparent from the graph (Figure 9) that the maximum level of cyanide was not attained after 30 days, this diffussion method in acid condition underestimated the actual cyanide potential of

cassava, jojoba meal and almond meal.

The liberation of cyanide resulting from acid treatment was further verified by comparing the spectrophotometric scan of the cyanide absorbing solutions obtained from cassava, deoiled jojoba meal and almond meal after 24 hours incubation time. Also, the spectrophotometric scan of the color developed in the colorimetric determination of cyanide was verified and maximum absorbance peaks compared. Figure 10 shows the spectra of the cyanide absorbing solution obtained from cassava, jojoba meal and almond meal before and after addition of acetic acid. All three samples showed a maximum absorption at 224 nm before addition of acetic acid and a small peak at 242 nm after addition of acetic acid. This further confirms the results from previous tests (Figures 6 and 8) indicating the liberation of cyanide from jojoba meal. The data shown in Figure 11 compare the scan of the color developed in the determination of cyanide in 1.0 N NaOH absorbing solution obtained from the above tests. The NaOH absorbing solution obtained from cassava, jojoba meal, jojoba meal plus KCN and almond meal gave a positive color reaction for cyanide with maximum absorbance at 580-585 nm.

Effect of Alkali-Heat Treatment on the Formation of Cyanide in Deoiled jojoba Meal. Our preliminary qualitative tests performed on aqueous extract showed positive reaction to cyanide color reagent when the extract was treated with NaOH and heat prior to the development of color. This

procedure was pursued to determine its applicability in the determination of cyanide in jojoba meal. Figure 12 show the effect of heating time in boiling water bath on the the cyanide levels determined from water extracts of deoiled jojoba meals. Maximum cyanide levels were attained after 2 minute heating in boiling water bath. The levels of cyanide in the two deoiled jojoba meals were considerably higher than the values obtained in the combined diffusion and acid treatment. A maximum of 1548 and 1121 ppm of cyanide were detected on deoiled jojoba meals from Sigma Chem. Co. and from commercial jojoba oil processing plant, respectively.

To confirm the positive color reaction from the NaOH-heat treatment of the water extract, tests were conducted on pure simmondsin and on crude isolates of simmondsin obtained from thin layer chromatography of freeze dried water extract and dried acetone extract of deoiled jojoba meal. Spectrophotometric scan of pure simmondsin and simmondsin isolate from TLC of acetone and freeze dried water extracts in 1.O N NaOH prior to heating are shown in Figure 13. All three samples showed maximum absorption peak at 224-225 nm. Heating the basic extract for 2 min in the boiling water bath resulted in formation of two additional peaks with maximum absorption at 291-291 and 212-213 nm (Figure 14). There was no apparent difference in the spectrophotometric spectra of pure simmondsin and that of simmondsin isolate obtained by thin layer chromatography. The spectra of the color developed after NaOH-heat

treatment of the extracts are shown in Figure 15. All three samples showed positive color reaction to cyanide with maximum absorbance at 582 nm. Based on these results, it is apparent that cyanide derivative is formed from simmondsin upon NaOH and heat treatment which reacts with the cyanide color reagent. Elliger et al. (1973) reported that basic degradation of simmondsin yielded 2-hydroxy-3-methoxyphenylacetonitrile. Nitrile compounds have been shown to form during the hydrolysis of linamarin in cassava and amygdalin in almond meal which are eventually hydrolyzed to form cyanide.

ACKNOWLEDGEMENTS. The authors gratefully acknowledge Mr. A. Verbiscar, Anver Bioscience Design Inc. for supplying pure simmondsin and Mr. Bill Watson of Desert King JMC. Ltd. for providing samples of jojoba seeds and meal.

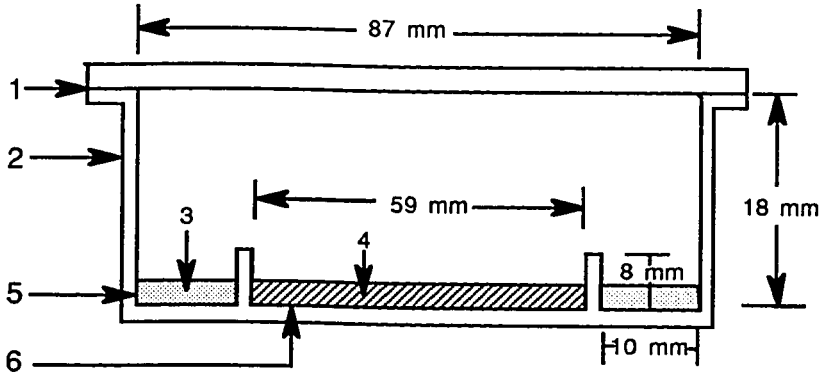


Figure 3. Schamatic diagram of Conway's semimicro diffusion apparatus. (1) Lid with silicon grease; (2) Diffusion chamber; (3) Absorbing solution (1.0 N NaOH); (4) Sample; (5) Outer chamber; (6) Inner chamber.

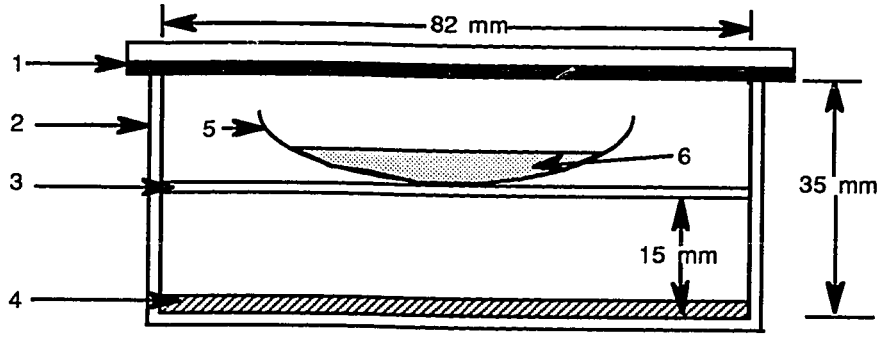


Figure 4. Schamatic diagram of modified semimacro diffusion apparatus. (1) Lid with silicon grease; (2) Diffusion chamber; (3) Glass rod for center well; (4) Sample; (5) Center well; (6) Absorbing solution (1.0 N NaOH).

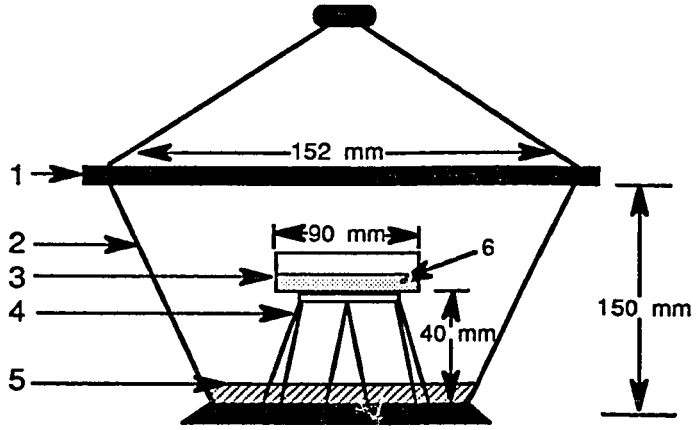


Figure 5. Schamatic diagram of modified macro diffusion apparatus. (1) Lid with silicon grease; (2) Diffusion chamber; (3) Center well; (4) Tripod stand for center well; (5) Sample; (6) Absorbing solution (1.0 N NaOH).

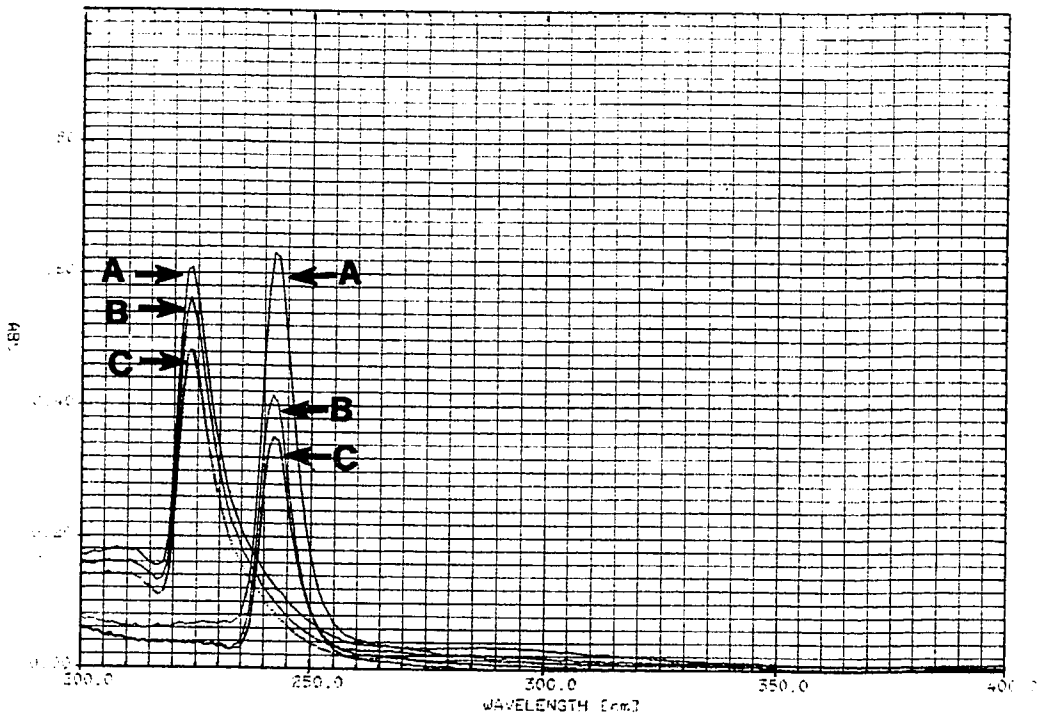


Figure 6. Absorbance spectra of the absorbing solution with and without treatment of 10.0 N acetic acid from hydrolysis of jojoba meal with acid (A), water (B), or phosphate buffer (pH 7.0) (C) after 48 hr of incubation.

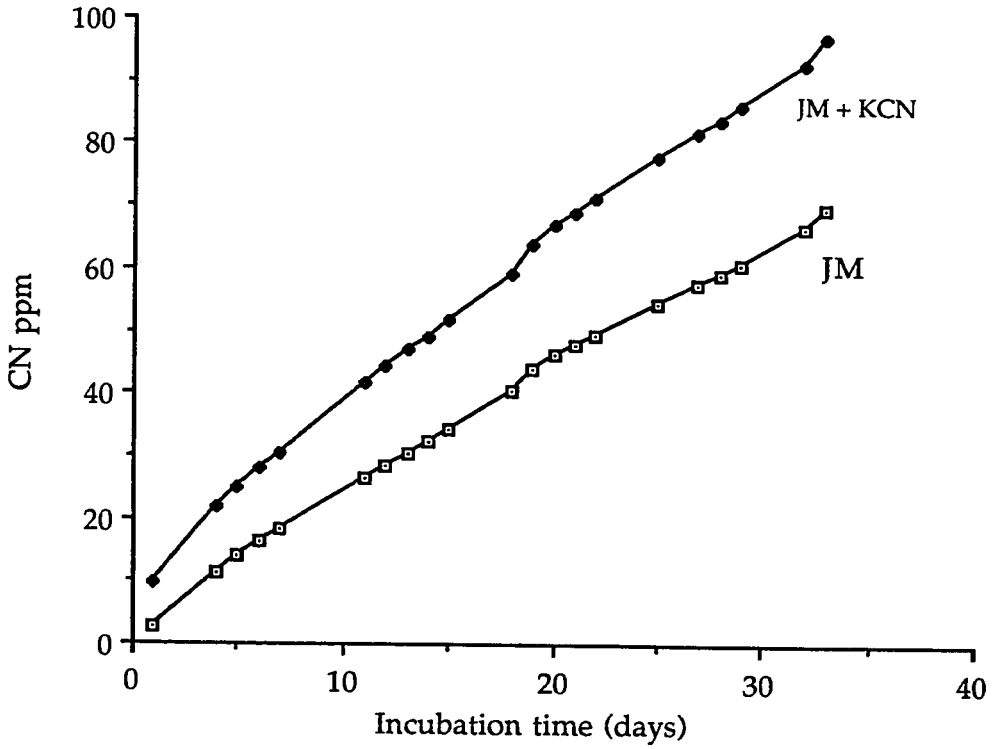


Figure 7. The effect of acid (1.0 N HCl) and incubation time on the liberation of cyanide from jojoba meal and KCN (potassium cyanide) added jojoba meal.

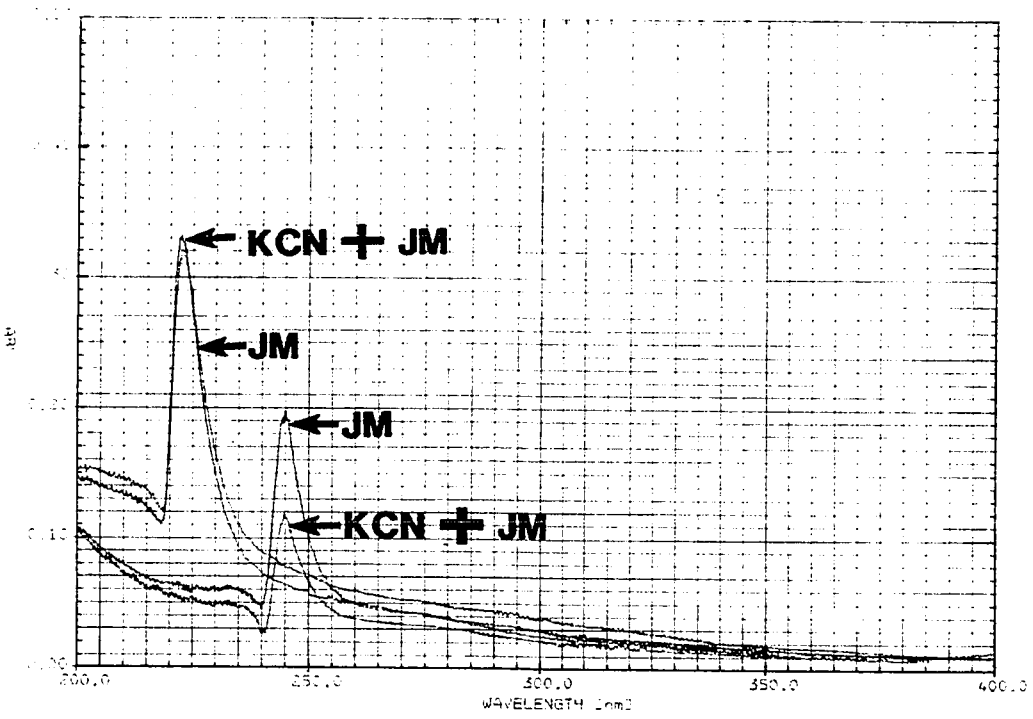


Figure 8. Absorbance spectra of the cyanide absorbing solution (1.0 N NaOH) with and without treatment of 10.0 N acetic acid from acid hydrolysis of jojoba meal and KCN added jojoba meal after 24 hr of incubation.

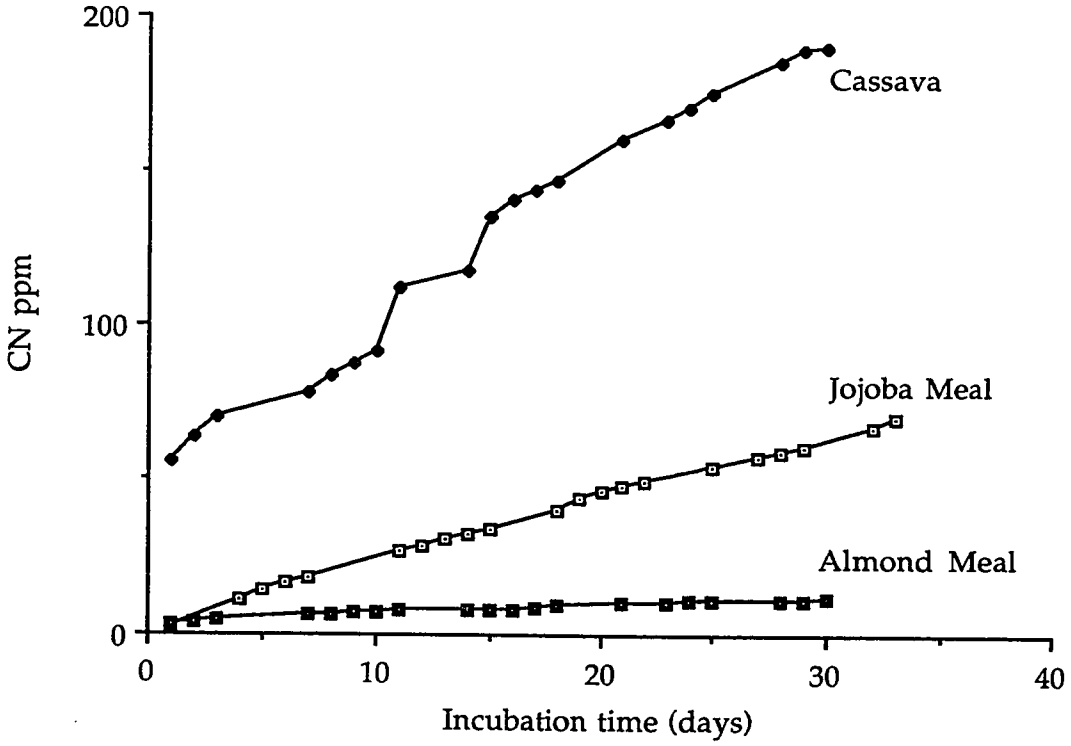


Figure 9. Comparision between the effect of acid on the liberation of cyanide from jojoba meal, cassava, and almond meal.

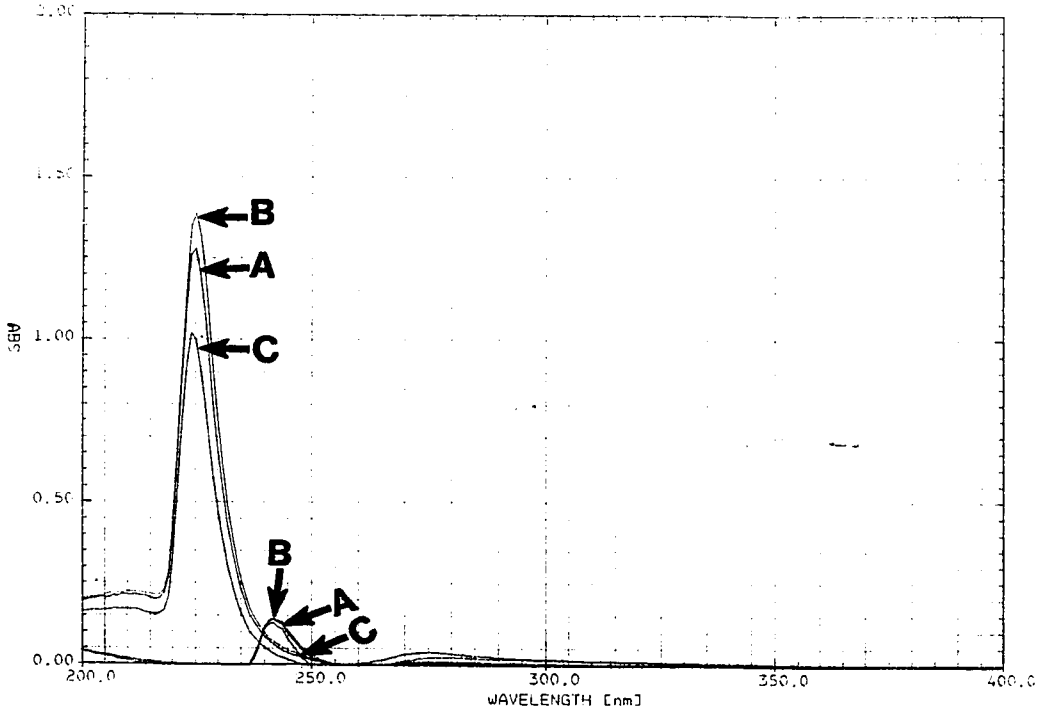


Figure 10. Comparision between the absorbance spectras of the cyanide absorbing solution with and without treatment of 10.0 N acetic acid from acid hydrolysis of jojoba meal (A), cassava (B), and almond meal (C) after 24 hr of incubation.

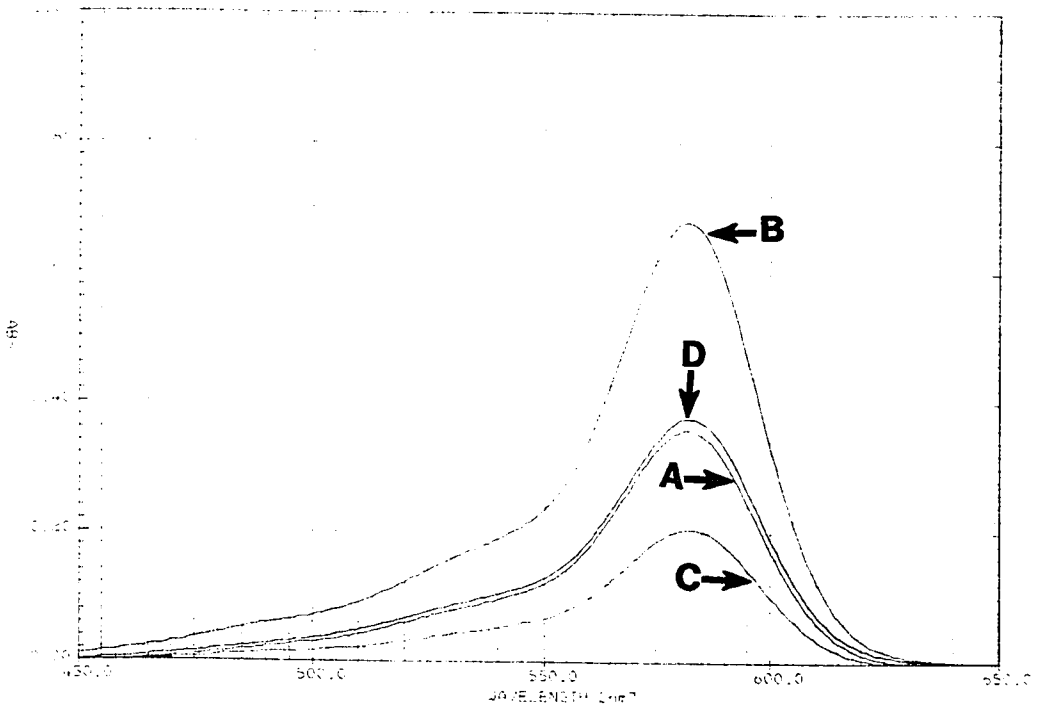


Figure 11. Comparision between absorbance spectras of the color of cyanide from acid hydrolysis of jojoba meal (A), cassava (B), almond meal (C), and KCN added jojoba meal (D) after 24 hr of incubation.

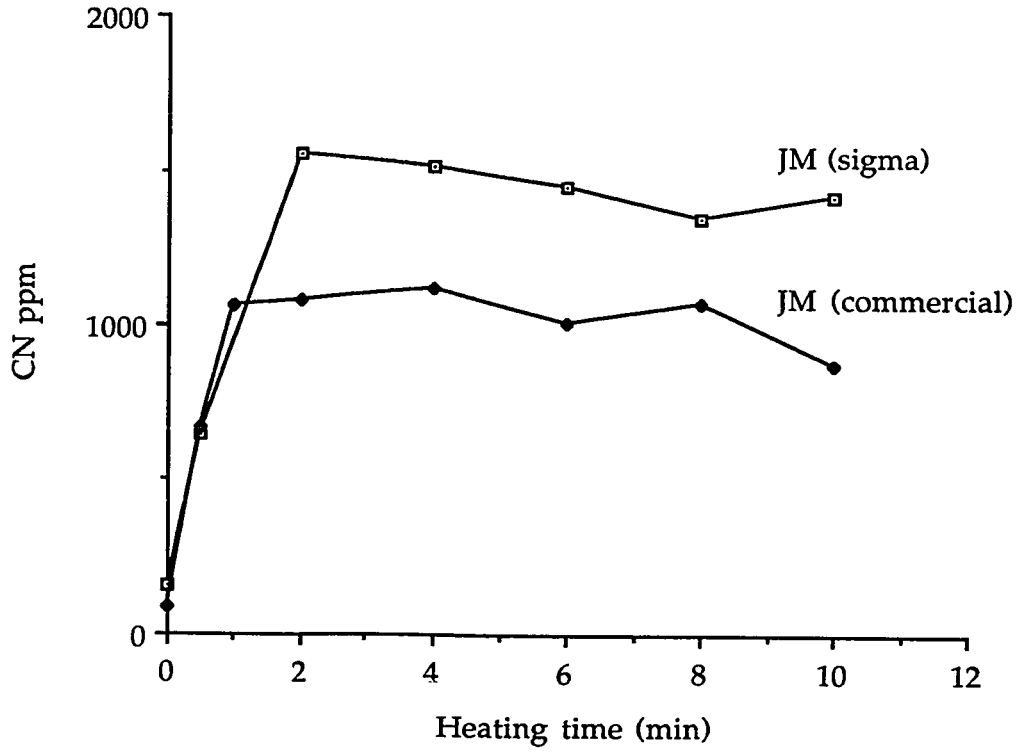


Figure 12. The effect of alkali-heat treatment on the formation of cyanide from jojoba meal.

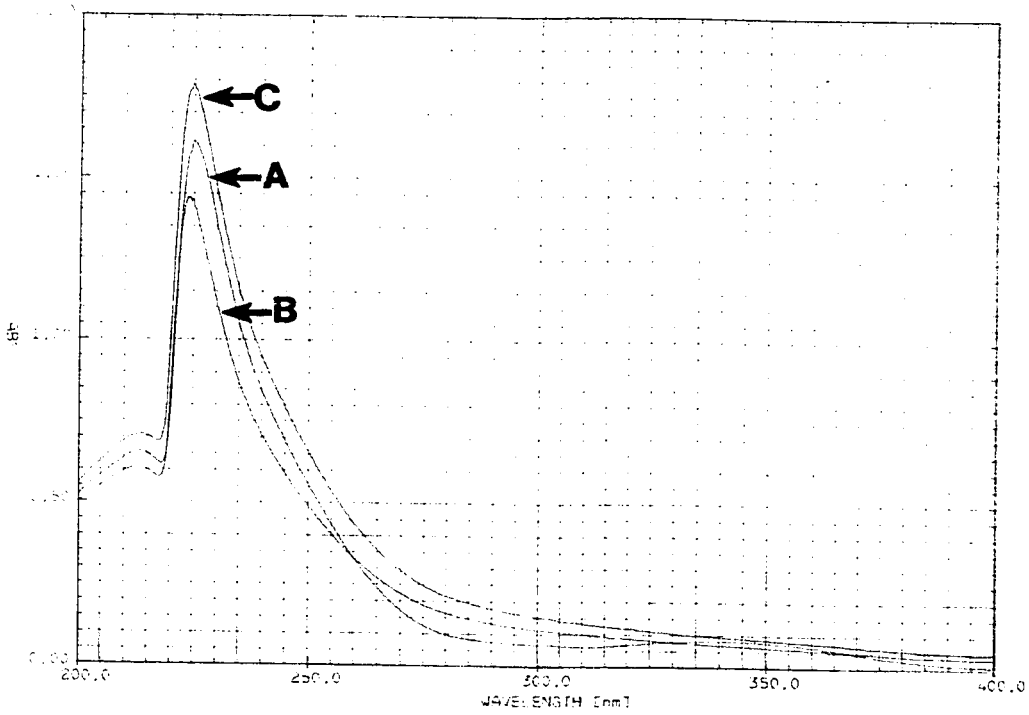


Figure 13. Absorbance spectra of pure simmondsin (A) and simmondsin isolated from freeze dried water extract (B) and acetone extract (C) of jojoba meal dissolved in 1.0 N NaOH.

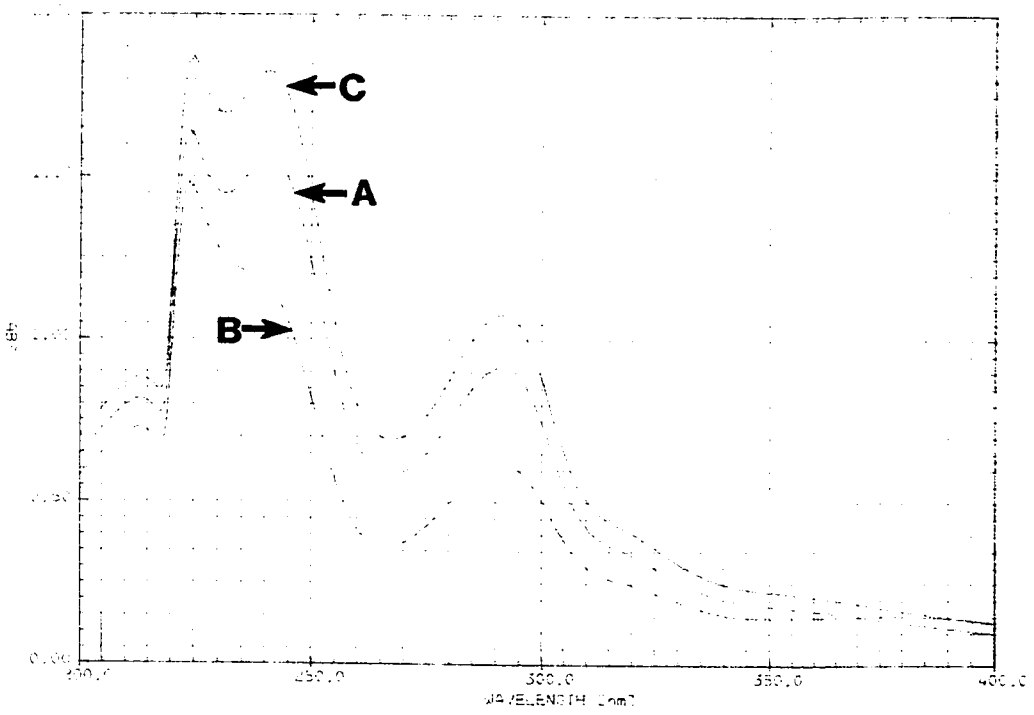


Figure 14. Absorbance spectra of pure simmondsin (A) and simmondsin isolated from freeze dried water extract (B) and acetone extract (C) of jojoba meal dissolved and heated with 1.0 N NaOH.

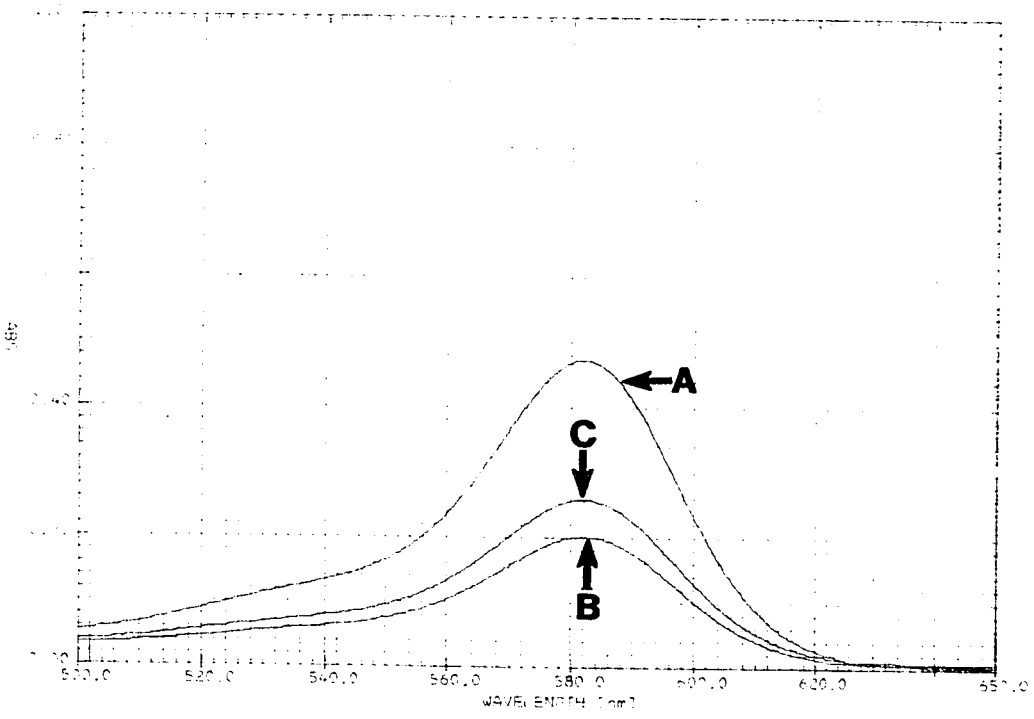


Figure 15. Absorbance spectra of the color of cyanide from pure simmondsin (A) and simmondsin isolated from freeze dried water extract (B) and acetone extract (C) of jojoba meal after heating with 1.0 N NaOH.

Table I. Proximate Analysis of the Whole Jojoba Seed (WJC) and Jojoba Meal Samples.

Constituent	WJS (a)	JM-1[b] (a)	JM-2[c] (a)
moisture (%)	2.40 ± 0.50	8.60 ± 0.60	4.20 ± 0.35
crude protein (%)	11.42 ± 0.17	27.85 ± 0.21	24.20 ± 0.08
crude fat (%)	48.00 ± 2.83	6.89 ± 0.17	1.10 ± 0.16
ash (%)	1.40 ± 0.12	3.88 ± 0.05	4.15 ± 0.07

(a) mean ± standard deviation of two determination.

(b) jojoba meal from sigma chemical company.

(c) jojoba meal from commercial oil processing plant.

Table II. The Cyanide Liberated from Jojoba Meal, Cassava, and Almond Meal After 24 hr Under Aqueous Condition.

Sample	Cyanide (ppm-dry basis)
Jojoba Meal	6.10
Cassava	13.42
Almond Meal	3.95

Table III. The Liberation of Cyanide from Jojoba Meal After 48 hr Incubated With Water, Acid (1.0 N HCl), or Phosphate Buffer (pH 7.0).

Sample	Cyanide (ppm-dry basis)
JM + Water	11.00
JM + Acid	63.90
JM + Buffer	5.25

LITERATURE CITED

AOAC. *Official Methods of Analysis, 13th ed.* Association of Official Analytical Chemists: Washington, DC, 1984.

Booth, A.; Elliger, C.; Waiss, A. Isolation of toxic factor from jojoba meal. *Life Sci.* 1974, 15, 1115-1120.

Cardoso, F. A.; Price, R. L. Extraction, characterization and functional properties of jojoba proteins. *IV. Reunion International de la Jojoba,* 1980, 305-316.

Conn, E. E. Cyanogenic glycosides. *J. Agri. Food Chem.* 1969, 17, 519-526.

Egli, K. L. Colorimetric Determination of Cyanide Liberated from Apricot Kernals. *JAOAC.* 1977. 60(4), 954-956.

Elliger, C.; Waiss, A. C. Jr; Lundin, R. E. Simmondsin, an unusual 2-cyanomethyleneclohexyl glucoside from Simmondsia californica. *J. Chem. Soc. Perkin Trans I.* 1973. 19, 2209-2213.

Fukuba, H.; Mendoza, E. Determination of cyanide in cassava. *Tropical Root Crops: Posthar. Physio. Proce.* 1984, 171-181.

Fukuba, H.; Igarashi, O.; Briones, C. M.; Mendoza, E. M. T. *Tropical Root Crops: Posthar. Physio. Proce.* 1984, 225-234.

Haisman, D. R.; Knight, D. J. *Bioche. Jour.* 1967, 103, 528-534.

Haskins, F.; Gorz, H.; Hill, R. Colorimetric determination of cyanide in enzyme-hydrolyzed extracts of dried sorghum leaves. *J. Agri. Food*

Chem. 1988, 36, 775-778.

Honig, D. H.; Hockridge, M. E.; Gould, R. M.; Rackis, J. J. Determination of cyanide in soybeans and soybeans products. *J. Agri. Food Chem.* 1983, 31, 272-275.

Lambert, J. L.; Ramasamy, J.; Paukstelis, J. V. Stable reagents for the colorimetric determination of cyanide by modified Konig reactions. *Anal. Chem.* 1975, 47, 916-918.

Maduagwa, E. N.; Umoh, I. B. *Toxico. Lett.* 1982, 10, 245.

Mandenius, C. F.; Bulow, L.; Danielsson, B. Determination of amygdalin and cyanide in industrial food samples using enzymic method. *Acta Chem. Scand.* 1983, B 37, 739.

Manos, C. G.; Schrynemeeckers, P. J.; Hogne, D. E.; Telford, J. N.; Stoewsand, G. S.; Beerman, D. H.; Babish, J. G.; Blue, J. T.; Shane, B. S.; J. Lisk, J. D. Toxicologic studied with lambs fed jojoba meal supplemented rations. *J. Agri. Food Chem.* 1986, 34, 801-805.

Nashida, T.; Shiraishi, T.; Uda, Y. Enzymatic analysis of cyanogenic glycosides II. A simple method by using a microdiffusion apparatus. *Chem. Pharm. Bull.* 1988, 36(1), 249-253.

National Academy of Science. 1975. *Products from Jojoba: A Promising New Crop for Arid Land.* National Academy Press. Washington, DC.

Ngoupayou, J. D. N. *Ph. D. Dissertation,* University of Arizona, 1982.

Padmaja, G. Evaluation of techniques to reduce assayable tannin and

cyanide in cassava leaves. *J. Agric. Food Chem.* 1989, 37, 712-716.

Seigler, D. S. Isolation and characterization of naturally occuring cyanogenic compounds. *Phytochem.* 1975, 14, 9-29.

Tinay, A. H. EL.; Bureng, P. L.; Yas, E. A. E. Hydrocyanic acid levels in fermented cassava. *J. Food Tech.* 1984, 19, 197-202.

Verbiscar, A. J.; Banigan, T. F. Composition of jojoba seed and foliage. *J. Agric. Food Chem.* 1978, 26, 1456-1459.

Verbiscar, A. J.; Banigan, T. F.; Weber, C. W.; Reid, B. L.; Trei, J. E.; Nelson, E. A. Detoxification and analysis of jojoba meal. *In Proc: 3rd International Conference on Jojoba*. 1978, 185.

Verbiscar, A. J.; Banigan, T. F.; Weber, C. W.; Reid, B. L.; Swingle, R. S.; Trie, J. E.; Nelson E. A. Detoxification of jojoba meal by lactobacilli. *J. Agri. Food Chem.* 1981, 29, 296-302.

Verbiscar, A. J.; Banigan, T. F.; Weber, C. W.; Reid, B. L.; Trei, J. E.; Nelson, E. A.; Raffauf, R. F.; Kosersky, D. Detoxification of jojoba meal. *J. Agri. Food Chem.* 1980, 28, 571-579.

Verbiscar, A. J.; Banigan, T. F. Jojoba seed meal as an animal feed. *JAOCS*. 1983, 60, 745.

Wiseman, M. O.; Price, R. L. Functional properties of protein concentrates from pressed jojoba meal. *Cereal Chem.* 1987, 64, 94-97.

Wisniak, J. Jojoba oil and derivatives. *Prog. in Chem. Fats and Other Lipids.* 1977, 15, 167.

Yermanos, D. M.; Duncan, S. J. Quantitative and qualitative characteristic of jojoba seed. *JAOCS.* 53, 80-82.

CHAPTER 3

SUMMARY AND RECOMMENDATIONS

Summary

Defatted jojoba meals prepared from whole seed, meal and commercially processed meal were used in the detection of cyanogenesis and in the determination of cyanide. Results of cyanogenesis tests were compared to cassava and almond meal. Whole seed meal prepared in the laboratory contained 22.50% protein. Deoiled jojoba meal protein content ranged from 25.0 to 32.0%. All protein values were computed on a dry, oil-free basis except for whole seed.

Preliminary study of the liberation of cyanide (cyanogenesis) from deoiled jojoba meal using semimicro diffusion apparatus (Nashida et al., 1988; Rubio et al., 1987) revealed that the method was not efficient enough to detect lower levels of cyanide. Because of this reason a macro diffusion apparatus was developed which showed that cyanide could be detected at lower levels using larger amounts of the sample.

Amount of cyanide liberated from deoiled jojoba meal using semi macro diffusion apparatus under aqueous conditions was almost tiwce that of almond meal, but was about half that liberated from cassava. Higher liberation of cyanide from jojoba meal was observed under acid conditions than that maintained under aqueous and phosphate buffer (pH 7.0) conditions. It is postulated that the maximum absorbance peak at 242 nm

obtained from the spectra of the cyanide absorbing solution (1.0 N NaOH) was due to the nitrile chromophore present in the cyanide compound liberated from deoiled jojoba meal samples.

The cyanogenesis test using macro diffusion apparatus under acid conditions carried out for 33 days at room temperature showed a relatively slow liberation rate of cyanide which indicated that this procedure cannot be used to determine the total cyanide content of jojoba meal. On a dry-weight basis, a total of 97.0 and 70.0 ppm cyanide were detected from jojoba meal with or without KCN after a 33 days incubation period, respectively. These contents exceeded the amounts which were compared found in soybean and soybean products (Honig et al., 1983). The spectra of color of cyanide of the absorbing solution obtained from jojoba meal, cassava, and almond meal under acid conditions proved that cyanogenesis occurred in jojoba meal.

The heating of water extract of jojoba meal with 1.0 N NaOH caused a high level of cyanide. This method for the determination of cyanide was easy, short, and reproducible and measured total content of cyanide in jojoba meal. Total cyanide content of deoiled jojoba meal determined by this method ranged from 1121 to 1548 ppm. These values were higher than the values obtained in the combined diffusion and acid treatment. Chemical treatment (NaOH and ammonia) had been found to retain a greater amount of cyanide as compared to direct drying of cassava leaf blades at 60° C (Padmaja, 1989).

The positive color reaction of cyanide obtained from water extract of jojoba meal treated with alkali (1.0 N NaOH) and heat lead the author to investigate whether this color was due to the cyanogenic glycosides (simmondsin) present in jojoba meal, the isolation of simmondsin and alkali-heat treatment was conducted. The results were compared to pure simmondsin. There was no apparent difference between the spectrophotometric spectra of pure simmondsin and isolated simmondsin by TLC. The positive color of cyanide obtained from pure simmondsin and isolated simmondsin shows that cyanide was formed from water extract of jojoba meal during alkali-heat treatment. The alkali degradation of simmondsin has been shown to produce 2-hydroxy-3-methoxyphenylacetonitrile (Elliger et al., 1973), although the effect of heat treatment has not been studied.

It is encouraging to note that the modified macro diffusion method reported in this study was found to evaluate cyanide content successfully at low levels in the detoxified jojoba meal. The alkali-heat treatment was also effective in the determination of total cyanide content of jojoba meal.

Recommendations

Although cyanide in jojoba meal is high, the nutritionally harmful effects of cyanide should be of concern when utilizing the meal as animal feed. Since acid hydrolysis of jojoba meal produced cyanide up to 33 days of diffusion, further study can be done by extending incubation time to get zero

level of cyanide. The extent of hydrolysis of jojoba meal can be studied by varying the acid concentrations. Animal studies can be done to assess the effect of acid hydrolyzed jojoba meal on animal growth. It is postulated that the detoxification of jojoba meal by acid will improve the feed quality of jojoba meal but animal studies are needed to test this hypothesis. Water and buffer hydrolysis had a effect on the liberation of cyanide from jojoba meal. Formation of cyanide from cassava and almond meal under water condition have been attributed to the hydrolysis of cyanogenic glycosides by the endogenous enzymes (Fukuba et al., 1984; Haisman & Knight, 1967). However, whether the cyanide detected is the result of endogenous enzyme hydrolysis of the cyanogenic glycosides in deoiled jojoba meal remains to be determined. Water extraction reduced simmondsin levels and improved the palatability of jojoba meal for livestock (Verbiscar et al., 1980). The study of the effect of acid on the liberation of cyanide from the freeze dried water extract (FDWE) of jojoba meal is needed. The NaOH-heat treatment on the formation of cyanide is also needed. More research is needed on the large-scale extraction of jojoba meal with water and other solvents and feeding experiments on large mammals. The identification of other cyanogenic glycosides than simmondsin is needed. Further research on combined alkali-heat treatment using different concentration of NaOH is recommended.

References

Association of Official Analytical Chemists (AOAC) (1984). Official Methods of Analysis (13th ed.). Washington, DC: AOAC.

Banigan, T. F., & Verbiscar, A. J. (1980). Detoxification of botanical foodstuffs. U.S. Patent, 4,209,539, June 24, U.S.A Department of Agriculture.

Booth, A. N., Elliger, C. A., & Waiss, A. C., Jr. (1974). Isolation of toxic factor from jojoba meal. Life Science, 15, 1115-1120.

Cardoso, F. A., & Price, R. L. (1980). Extraction, characterization and functional properties of jojoba proteins. IV Reunion International de la Jojoba, 305-316.

Conn, E. E. (1969). Cyanogenic glycosides. Journal of Agricultural and Food Chemistry, 17, 519-526.

Conn, E. E. (1971). Cyanogens. In Toxic occuring naturally in foods, National Acadamy of Science, Washington, D.C.

Cotgageorge, A. G. (1978). Detoxification of jojoba. Unpublished master's thesis, The University of Arizona, Tuscon, AZ.

Egli, K. L. (1977). Colorimetric determinationof cyanide liberated from apricot kernals. Journal of the Association of Official Analytical Chemists, 60(4), 954-956.

Elliger, C. A., Waiss, A. C. Jr., & Lundin, R. E. (1973). Simmondsin, an

unusual 2-cyanomethylenecyclohexyl glucoside from Simmondsia californica. Journal of the Chemical Society. Perkin Transdines I 19: 2209-2213.

Elliger, C. A., Waiss, C. A. Jr., & Booth, A. N. (1975). Detoxification of Jojoba meal. U. S. Patent, 3,919,432, November 11, U. S. A. Department of Agriculture.

Fukuba, H., & Mendoza, E. M. T. (1984). Determination of cyanide in cassava. Tropical Root crops: Postharvset Physiology and Processing, pp. 171-18 .

Fukuba, H., Igarashi, O., Briones, C. M., & Mendoza, E. M. T. (1984). Cyanogenic glycosides in cassava and cassava products: Determination and detoxification. Tropical Root Crops: Postharvest Physiology and Processing. pp. 225-234.

Haisman, D. R., & Knight, D. J. (1967). The enzymatic hydrolysis of amygdalin. Biochemical Journal, 103, 528-534.

Harris, J. R., Merson, G. H. J., Hardy, M. J., & Curtis, D. J. (1980). Determination of cyanide in animal feeding stuffs. Analyst, 105, 974-980.

Haskins, F. A., Gorz, H. J., & Hill, R. M. (1988). Colorimetric determination of cyanide in enzyme-hydrolyzed extracts of dried sorghum leaves. Journal of Agricultural and Food Chemistry, 36, 775-778.

Hinman, W. C. (1986). Potential new crops. Scientific American, 255, 32-

37.

Honig, D. H., Hockridge, M. E., Gould, R. M., & Rackis, J. J. (1983). Determination of cyanide in soybeans and soybeans products. Journal of Agricultural and Food Chemistry, 31, 272-27 .

Ikediobi, C. O., Onyia, G. O. C., & Eluwah, C. E. (1980). A rapid and enexpensive enzymatic assay for total cyanide in cassava (*Manihot esculenta Crantz*) and cassava products. Journal of Agricultural Biological Chemistry, 44 (12), 2803-2809.

Lambert, J. L., Ramasamy, J., & Paukstelis, J. V. (1975). Stable reagents for the colorimetric determination of cyanide by modified Konig reactions. Analytical Chemistry, 47, 916-918.

Maduagwa, E. N., & Umoh, I. B. (1982). Detoxification of cassava leaves by simple traditional methods. Toxicology Letters, 10, 245-248.

Mandenius, C. F., Bulow, L., & Danielsson, B. (1983). Determination of amygdalin and cyanide in industrial food samples using enzymic method. Acta Chemica Scandinavica, B 37 (8), 739-742.

Manos, C. G., Schrynemeeckers, P. J., Hogne, D. E., Telford, J. N., Stoewsand, G. S., Beerman, D. H., Babish, J. G., Blue, J. T., Shane, B. S., & Lisk, D. J. (1986). Toxicologic studies with lambs fed jojoba meal supplemented rations. Journal of Agricultural and Food Chemistry, 34, 801-805.

Mendoza, E. M. T., Kojima, M., Iwatsuki, N., Fukuba, H., & Uritani, I.

(1984). Evaluation of some methods for the analysis of cyanide in cassava. Tropical Root Crops: Postharvest Physiology and Processing. pp. 235-242.

Nahrstedt, A., Erb, N., & Zinsmeister, H-D. (1981). Methods of liberating and estimating hydrocyanic acid from cyanogenic plant material. In: B. Vennesland, E. E. Conn, J. Christopher, J. Westley, & F. Wissing (Eds.), Cyanide in biology (pp. 461-471). London and New York: Academic Press.

Nashida, T., Shiraishi, T., & Uda, Y. (1988). Enzymatic analysis of cyanogenic glycosides II. A simple method by using a microdiffusion apparatus. Chemical & Pharmaceutical Bulletin (Tokyo), 36 (1) 249-253.

National Academy of Science. (1975). Products from jojoba: A Promising new crop for arid lands. Washington, DC: National Academy Press.

National Academy of Science. (1977). Jojoba: Feasability for cultivation on indian reservation in the sonoran desert region. Washington, DC: National Academy Press.

National Academy of Science. (1985). Jojoba: New crop for arid lands, new raw material for industry. Washington, DC: National Academy Press.

Ngoupayou, J. D. N. (1982). Nutritional evaluation of jojoba meal. Unpublished pH. D. Dissertation, The University of Arizona, Tuscon, AZ.

Padmaja, G. (1989). Evaluation of techniques to rreduce assayable tannin

and cyanide in cassava leaves. Journal of Agricultural and Food Chemistry, 37, 712-716.

Rubio, R., Sanz, J., & Rauret, G. (1987). Determination of cyanide using a microdiffusion technique and poteiometric measurement. Analyst, 112, 1705-1708.

Seigler, D. S. (1975). Isolation and characterization of Naturally occuring cyanogenic compounds. Phytochemistry, 14, 9-29.

Tinay, A. H. E. L., Bureng, P. L., & Yas, E. A. E. (1984). Hydrocyanic acid levels in fermented cassava. Journal of Food Technology, 19, 197-202.

Verbiscar, A. J., & Banigan, T. F. (1978). Composition of jojoba seed and foliage. Journal of Agricultural and Food Chemistry, 26, 1456-1459.

Verbiscar, A. J., Banigan, T. F., Weber, C. W., Reid, B. L., Trei, J. E., & Nelson, E. A. (1978). Detoxification and analyses of jojoba meal. Proceedings of the Third International Conference on Jojoba. (pp. 185-197).

Verbiscar, A. J., Banigan, T. F., Weber, C. W., Reid, B. L., Swingle, R. S., Trei, J. E., & Nelson, E. A. (1981). Detoxification of jojoba meal by lactobacilli. Journal of Agricultural and Food Chemistry, 29, 296-302.

Verbiscar, A. J., Banigan, T. F., Weber, C. W., Reid, B. L., Trei, J. E., Nelson, E. A., Raffauf, R. F., & Kosersky, D. (1980). Detoxification of jojoba meal. Journal of Agricultural and Food Chemistry, 28, 571-578.

Verbiscar, A. J., & Banigan, T. F. (1983). Jojoba seed meal as an animal

feed. The Journal of the American Oil Chemical Society, 60, 74.

Weber, C. W. (1973). Jojoba meal and wax fed to mice. Jojoba Happenings, 5, 11-13.

Wiseman, M. O., & Price, R. L. (1987). Functional proprites of protein concentrates from pressed jojoba meal. Cereal Chemistry, 64 (2), 94-97.

Wisniak, J. (1977). Jojoba oil and derivatives. Progress in Chemistry. Fats and other Lipids, 28, 167-218.

Yermanos, D. M., & Duncan, C. C. (1976). Quantitative and qualitative characteristic of jojoba seed. Journal of the American Oil Chemists Society, 53, 80-82.